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SCIENCE DIRECT
Field Crops Research 96 (2006) 224–234
Field Crops Research
www.elsevier.com/locate/fcr

Positional differences in nitrogen and sugar concentrations of upper leaves relate to plant N status in rice under different N rates

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Received 1 July 2005; accepted 2 July 2005

Abstract

This research was undertaken to investigate the spatial differences of protein-N, non-protein-N, total N, soluble sugar and starch concentrations in the four upper fully expanded leaves, and their relationships with plant N concentration in rice (*Oryza sativa*). Plant and leaf samples were analyzed for concentrations of N and C components from the field experiments with six rice genotypes and 5 N fertilization rates in two growing seasons at Nanjing, China. The results showed that protein-N, non-protein-N and total N concentrations in rice leaves of all genotypes increased significantly with increasing N rates, and they could be all used as indices for diagnosis of plant nitrogen nutrition. Leaves at different positions of all genotypes responded differentially to the increasing N rates. The differences in each chemical component of the leaf tissue were smaller among the first, second and third leaf from the top (1–3 LFT), but greater between the 4 LFT and the 1–3 LFT from the top. The differences between the 4 LFT and the 1–3 LFT in tissue nitrogen of all genotypes decreased with increasing N rates, and could be well characterized by the relative positional difference index (PDI) between the 4 LFT and the 1–3 LFT. The PDI indices were all exponentially related to plant nitrogen concentrations, which could be grouped into two regression curves for Japonica rice and Indica rice, respectively. In particular, the PDI between 4 and 3 LFT could accurately indicate plant nitrogen status of rice genotypes under varied nitrogen supply levels over different growth stages in two growing seasons. It is concluded that the leaf positional difference index in relation to N concentration between the third and fourth leaves should be ideal indicator of plant nitrogen status in rice.
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Keywords: Rice; Genotypes; Nitrogen rates; Plant nitrogen diagnosis; Leaf positional difference index (PDI)

1. Introduction

Nitrogen (N) is one of the most important plant nutrients and plays a vital role in plant photosynthesis and biomass production. During the past years, a great deal of research has been undertaken on physiology, ecology and management of nitrogen nutrition in crop plants (Abrol et al., 1999). Recommendation of N fertilization strategy and improvement of N management efficiency heavily rely on precise evaluation of nitrogen status in plant–soil systems (Costa et al., 2001). Since plant nitrogen status is an integrated indication of soil nitrogen supply, and plant nitrogen uptake and demand (Balasubramanian et al., 1999), diagnosis of plant N is considered as a key technique for recommending the time and quantity of N fertilization in crop production (Li et al., 2003).

Total nitrogen concentration is the traditional plant nitrogen index and has been studied extensively with different crops. Total plant N is closely associated with yield performance, and thus has become a widely used diagnosis index, with critical N concentrations established at different growth stages of main crops (Leight and Johnson, 1985). Several studies show that when there is a light N deficit within plants, the demands for NO_3^-–N, free amino acid and free amino nitrogen will increase quickly, without necessarily a simultaneous marked change in total nitrogen. Therefore, the changes in these components could be used to evaluate plant nitrogen status and recommend fertilization strategy (Roth and Fox, 1989; Yuan, 1989; Zhang et al., 1996). Since there is association between plant nitrogen metabolism and carbon metabolism, plant starch concentration and C/N ratio could also be used to indicate the status of

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doi:10.1016/j.fcr.2005.07.008

plant nitrogen nutrition in crops (Lee et al., 1989; Zou et al., 2001).

Several methods for non-destructive estimation of plant nitrogen nutrition have been proposed, assuming that plant nitrogen status can be determined by leaf color charts, chlorophyll meter (SPAD), reflectance spectra, laser data and chlorophyll fluorescence (Filella et al., 1995; Kon et al., 1999; Ntamatungiro et al., 1999; Johnkutty et al., 2000). These diagnosis indices of plant nitrogen nutrition have been tested and used for recommending N fertilization in different crops. Normally, the first or second upper fully expanded leaf is sampled to determine these indices (Wallihan and Moomaw, 1967; Peng et al., 1996), yet there are some differences in the SPAD critical indices for recommending fertilization among different varieties or different growth stages of the same variety in rice (Zhang et al., 2003). Recent studies show that there are large differences in sensitivity of response to increased N rates between the upper and lower leaves in rice (Zhou and Wang, 2003), and the ratio of SPAD readings between different leaf positions is closely correlated with plant nitrogen concentration (Wang et al., 2002a). Other studies also suggested using the SPAD reading ratio as a nitrogen nutrition diagnosis index of rice in order to overcome the problem of inconsistent SPAD critical indices in N fertilizer recommendation for various varieties or different growth stages of the same variety (Shen et al., 2002). However, these studies did not explore the differences in tissue concentrations of nitrogen and carbon components in different positional leaves and their relationships with plant nitrogen concentration. Thus, more information is needed about the proper sample leaf position and biochemical index for diagnosis of plant nitrogen nutrition in rice.

The objectives of this research were to characterize the spatial differences in tissue concentrations of nitrogen and carbon components of the four upper fully expanded leaves under different N rates and genotypes, and to obtain the suitable sample leaf position and tissue chemical index for reliable diagnosis of plant nitrogen status in rice.

2. Materials and methods

2.1. Field experiments

Two duplicated experiments were carried out at the Experiment Station of Nanjing Agricultural University (32°03′N, 118°48′E) during the growing seasons of 2001 and 2002, each involving N rates and genotypes. The soil type was Gleyed paddy soil with pH 6.5, organic matter of 12.7 g kg^{-1} and total N of 0.9 g kg^{-1} at 0–25 cm depth.

Five N rates of 0, 150, 250, 350 and 450 kg N ha^{-1} were established in the experiment, and designed as severe N deficiency, light N deficiency, normal N supply, light N excess and severe N excess, respectively. The form of N fertilizer was urea with N content of 46%. The distribution of total N amount at different growth stages was 50% before transplanting and 25% at panicle initiation and 25% at spikelet initiation, respectively, as with the recommended fertilization strategy in local rice growing region.

Six genotypes were used in the experiment to represent different growth characters and nitrogen responses. They were Wuxiangjing 9 (Japonica, about 160 days of growing period), 9915 (Japonica, about 155 days of growing period), Kimaze (Japonica, about 130 days of growing period), Koshihikari (Japonica, about 125 days of growing period), H97-322 (Indica, about 155 days of growing period) and Baidao (Indica, about 140 days of growing period).

The experiment was a two-factorial randomized complete block design with three replications and plot size of 13.5 m^2 (4.5 m × 3.0 m). The banks between the individual plots were covered with plastic film to prevent fertilizer penetration across the treatments. In 2001, the sowing date was May 9 and transplanting date was June 16, and in 2002 they were May 10 and June 17. Seedlings were raised by dry nursery and transplanted at 30 cm × 15 cm spacing and two seedlings per hill in both seasons.

2.2. Sampling and measurements

Plants were sampled from each plot at panicle initiation and heading stages. Before each sampling, tiller number of each hill within the plot was counted (except border rows) for average tiller number per hill. Then five hills of whole plants for each plot were sampled. After removing roots and panicles (after heading), the plant sample was put into a sample bag. Also, six plants near each sampling location were taken and the first, second, third and fourth upper fully expanded leaves were picked and put into different sample bags according to the leaf positions, respectively. All the fresh samples were placed in a forced-air oven, killed for an hour at 105 °C, and dried until they reached a constant weight at the temperature of 85 °C. The dried samples were milled to pass 1 mm screen, and then stored in plastic bags for chemical analysis.

Total plant nitrogen was determined by Kjeldahl method. Plant samples of 0.5 g were digested with 3 g of catalyst of 3:1 K_2SO_4:$CuSO_4$ for at least 6 h at 375 °C, along with 10 ml of H_2SO_4 and 2 ml H_2O_2. Leaf protein-N and non-protein-N were separated with 5% CCl_3COOH. Leaf sample of 0.5 g was mixed with 5% CCl_3COOH 40 ml and then shook for at least 1 h before centrifuging and filtering under room temperature (Li et al., 2000). The filtrate was concentrated to 10 ml for non-protein-N, and the residue for protein-N, both were determined by the Kjeldahl method.

The soluble sugars and starch were extracted with alcohol and HCl, respectively, and measured by anthrone solution colorimetric method (Cai and Yuan, 1982). The leaf of 0.1 g was boiled in 80% alcohol (10 ml) for 30 min at 80–85 °C and centrifuged to get the supernatant for assay of soluble sugars. The soluble sugars were extracted three times with this protocol. The residue was boiled in 2% HCl (125 ml) for at least 1 h until starch was hydrolyzed completely and then

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